**In vitro susceptibility of Plasmodium falciparum to monodesethylamodiaquine, quinine, mefloquine and halofantrine in Abidjan (Côte d’Ivoire)**

Yavo W 1,2, Bla KB 3, Djaman AJ 3,6, Assi SB 4, Basco LK 5, Mazabraud A 6, Koné M 1

1- Department of Parasitology and Mycology, Faculty of Pharmaceutical and Biological Sciences, University of Cocody-Abidjan
2- Laboratory of Microbiology, National Institute of Public Health of Côte d’Ivoire
3- Biochemical laboratory pharmacodynamic, Faculty of Biosciences, University of Cocody-Abidjan
4- Pierre Richet Institute of Bouaké, Côte d’Ivoire
5- Research Institute for Development, Malaria Research Unit 198, Yaoundé, Cameroon
6- Institute of integrative and cell biology of animals, UMR 8080, University Paris-Sud XI, Orsay, France

**Abstract**

**Background:** Malaria is the primary cause of hospitalization in Côte d’Ivoire. Early treatment is one of the strategies to control this illness. However, the spread of resistance of Plasmodium falciparum to antimalarial drugs can seriously compromise this strategy.

**Objectives:** The aim of this study was to assess the in vitro susceptibility of P. falciparum to monodesethylamodiaquine and aminoalcohols in Abidjan (Côte d’Ivoire).

**Methods:** We assessed the in vitro susceptibility of isolates collected from patients with uncomplicated malaria by using the WHO optical microtest technique.

**Results:** The proportions of resistance to monodesethylamodiaquine, méfloquine and halofantrine were 12.5%, 15.6% and 25.9%, respectively. For quinine, none of isolates showed evidence of in vitro resistance. However, two isolates (6.1%) had IC$_{50}$ values above 300 nM. The IC$_{50}$ of each drug was positively and significantly correlated to that of the other three drugs, and the correlation was higher between halofantrine and mefloquine.

**Conclusions:** Our results showed that the in vitro chloroquine resistance reported in previous studies has been extended to other antimalarial drugs investigated in this study except for quinine. Therefore, it is necessary to implement a long-term monitoring system of antimalarial drug resistance.

**Key words:** in vitro test, Plasmodium falciparum, monodesethylamodiaquine, quinine, mefloquine, halofantrine, Abidjan (Côte d’Ivoire).

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**Introduction**

Malaria still remains a major public health problem in sub-Saharan African countries 1. Several strategies, such as rapid diagnosis and appropriate treatment, have been recommended to control malaria. However, faced with the growing inefficiency of monotherapies, as in most countries, the Ministry of Public Health in Côte d’Ivoire has adopted a novel strategy based on the use of drug combinations including artemisinin derivatives (artemisinin-based combination therapy, i.e. ACT). Quinine is reserved for curative treatment in case of treatment failure of ACTs or severe and complicated malaria. Sulfadoxine-pyrimethamine (SP) is used to prevent malaria in pregnant women 3,7. Unfortunately, these recommendations are not always followed by drug prescriptors 4, thereby increasing the probability of selection and spread of drug-resistant Plasmodium falciparum strains. Because of the presence of mefloquine and amodiaquine in some ACTs, it is necessary to assess the susceptibility of P. falciparum to these antimalarial drugs by in vitro and/or in vivo tests.

Moreover, a decrease in the sensitivity of P. falciparum to quinine has been reported in Southeast Asia, East Africa and South America 5,6,7. As quinine has been used for decades to treat severe and complicated
malaria until now, resistance to quinine could lead to a public health disaster. It is therefore necessary to implement an improved program for monitoring drug-resistant malaria in order to plan and adopt appropriate strategies to control this disease. Several methods can be used to evaluate the susceptibility of *P. falciparum* to antimalarial drugs. Laboratory tools, such as *in vitro* drug sensitivity assays, can provide an early warning to orient therapeutic efficacy studies and antimalarial treatment policy.

Furthermore, cultivation of clinical isolates and measurement of their susceptibility to antimalarial compounds *in vitro* remove host-related variables, such as patients’ compliance, nutritional status, immune status, re-infection and pharmacokinetics, thereby providing a powerful technique for detecting the emergence of drug-resistant parasites. The aims of the present study were: a) to assess the *in vitro* susceptibility of clinical isolates of *P. falciparum* to monodesethylamodiaquine, quinine, mefloquine, and halofantrine, and b) analyse the potential for cross-resistance between these drugs.

**Methods**

**Study area**

The study was carried out between February 2006 and February 2007 in the district of Abobo, situated in the north of Abidjan (the economic capital city). In this area, malaria is hyperendemic with seasonal transmission. The most common vectors are *Anopheles gambiae* ss and *A. funestus*.

**Isolates of *P. falciparum***

Patients aged between 2 to 45 years presenting signs and symptoms of uncomplicated malaria were recruited at El Rapha and Anokoua Kouté, two health centers of Abobo area. Informed consent was obtained from the patients or guardian accompanying the sick children. The study was approved by the Ethics Committee of the Ivorian National Institute of Public Health (NIPH).

Venous blood samples were collected in EDTA-coated Vacutainer® tubes (Terumo Europe N.V., Leuven, Belgium) before treatment. They were transported at 4°C to NIPH within 6 h, if the parasitemia was at least 4,000 asexual parasites/µl of blood.

Parasitized erythrocytes were washed three times in RPMI 1640 medium (Invitrogen, UK), and Giemsa-stained thin blood smears were examined under the microscope to determine the parasite density and confirm the *Plasmodium* species (*P. falciparum* monoinfections).

Samples with parasitemia ranging from 0.1% to 0.25% were used directly to test drug susceptibility. If parasitemia exceeded 0.25%, infected erythrocytes were diluted to this parasitemia range with uninfected erythrocytes. Patients were treated with amodiaquine-arteusunate or artemether-lumefantrine according to the recommended national therapeutic regimens.

**Drugs**

The test compounds were obtained from the following sources: monodesethylamodiaquine (TDR/World Health Organization [WHO] Drug Discovery Research), quinine chlorhydrate (Sanofi-Aventis, Antony, France), mefloquine hydrochloride (Roche, Mannheim, Germany), and halofantrine (Glaxo Smith Kline, Evreux, France). Stock solutions of each drug were prepared in 70% methanol. Twofold serial dilutions were prepared in RPMI 1640 medium and distributed in triplicate into 96-well culture plates.

**In vitro assay**

The WHO microtest technique was used to measure the inhibition of schizont maturation by microscopy. Washed infected erythrocytes were suspended in RPMI 1640 with 10% human serum, 25 mM HEPES, and 25 mM NaHCO₃ at a hematocrit of 1.5%. Fifty microliters of the blood-medium mixture were distributed into each well of the predosed 96-well tissue culture plates and incubated at 37°C in candle jars for 48 h according to standard methodology. Final concentrations were ranged from 3.125 to 400 nM for monodesethylamodiaquine and mefloquine, from 12.5 to 1600 nM for quinine and from 0.25 to 32 nM for halofantrine. After incubation period, parasites were harvested and Giemsa stained thick blood films were prepared. The number of schizonts, defined as schizonts with more than 3 nuclei, was counted per 200 asexual parasites. Isolates with less than 20% of schizonts in drug-free control well were excluded. The results were expressed as 50% inhibitory concentration values (IC₅₀).

The cut-off values for in vitro resistance to monodesethylamodiaquine, quinine, mefloquine, halofantrine were fixed at 60 nM, 800 nM, 30 nM, and 6 nM respectively.
Statistical analysis

The IC\textsubscript{50} values were determined by nonlinear regression analysis of the plot of logarithm of concentration against growth inhibition. Data were adapted to fit the logprobit model (Excel; Microsoft, Redmond, WA). The in vitro response was expressed as the geometric mean IC\textsubscript{50} values with 95% confidence intervals.

The degree of correlation between different antimalarial drugs was estimated by the Spearman correlation coefficient (rho) and the coefficient of determination (r\textsuperscript{2}). The level of significance was set at 0.05.

Results

Forty three isolates of \textit{P. falciparum} were collected. In this study, asexual parasite densities ranged from 0.1% to 13.5%. The following proportions of isolates were successfully cultured for each drug tested: 74.4% \textit{(32/43)} for monodesethylamodiaquine, 76.7% \textit{(33/43)} for quinine, 76.7% \textit{(33/43)} for mefloquine and 62.8% \textit{(27/43)} for halofantrine. The geometric mean IC\textsubscript{50}s of four antimalarial drugs tested are summarized in Table 1.

**Table 1: The geometric mean IC\textsubscript{50}s of four antimalarial drugs tested against wild isolates of \textit{P. falciparum} in Abidjan**

<table>
<thead>
<tr>
<th>Drugs</th>
<th>No. of isolates tested</th>
<th>Geometric Mean IC\textsubscript{50} (nM)</th>
<th>95% Confidence interval (nM)</th>
<th>Range (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monodesethylamodiaquine</td>
<td>32</td>
<td>13.3</td>
<td>12 – 14.6</td>
<td>2.04 – 269</td>
</tr>
<tr>
<td>Quinine</td>
<td>33</td>
<td>60.6</td>
<td>59.6 – 61.7</td>
<td>4.31 – 486</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>33</td>
<td>7.45</td>
<td>6.41 – 8.49</td>
<td>1.11 – 64.2</td>
</tr>
<tr>
<td>Halofantrine</td>
<td>27</td>
<td>1.64</td>
<td>0.93 – 2.35</td>
<td>0.17 – 20.4</td>
</tr>
</tbody>
</table>

Four (12.5%) isolates were resistant to monodesethylamodiaquine, and five (15.6%) monodesethylamodiaquine-sensitive isolates showed IC\textsubscript{50} up to 25 nM. Five (15.2%) and seven (25.9%) isolates showed \textit{in vitro} resistance to mefloquine and halofantrine, respectively. For each antimalarial drug, there were three sensitive isolates which showed borderline sensitivity (i.e. IC\textsubscript{50} > 20 nM but < 30 nM for mefloquine and > 4 nM but < 6 nM for halofantrine). There was no resistance to quinine (Figure 1). Two isolates (6.1%) presented quinine IC\textsubscript{50} up to 300 nM.

Concerning cross-resistance, one isolate was resistant \textit{in vitro} to monodesethylamodiaquine, mefloquine and halofantrine, three isolates were resistant to monodesethylamodiaquine and halofantrine, and additional three isolates were resistant \textit{in vitro} to halofantrine and mefloquine.

The IC\textsubscript{50} of each drug was positively and significantly correlated to that of the other three drugs, and correlation was highest between halofantrine and mefloquine. Mefloquine and quinine IC\textsubscript{50}s were weakly correlated (Table 2).

**Table 2: Correlation between the IC\textsubscript{50}s values of the four antimalarial drugs tested**

<table>
<thead>
<tr>
<th>Drug pair</th>
<th>No. of isolates tested</th>
<th>r</th>
<th>r\textsuperscript{2}</th>
<th>p*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Monodesethylamodiaquine - Halofantrine</td>
<td>27</td>
<td>0.52</td>
<td>0.27</td>
<td>0.0003</td>
</tr>
<tr>
<td>Monodesethylamodiaquine - Quinine</td>
<td>32</td>
<td>0.56</td>
<td>0.32</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Monodesethylamodiaquine - Mefloquine</td>
<td>32</td>
<td>0.24</td>
<td>0.06</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Halofantrine - Quinine</td>
<td>27</td>
<td>0.32</td>
<td>0.10</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Halofantrine - Mefloquine</td>
<td>27</td>
<td>0.67</td>
<td>0.46</td>
<td>0.0039</td>
</tr>
<tr>
<td>Mefloquine - Quinine</td>
<td>33</td>
<td>0.16</td>
<td>0.03</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

*Student Fisher test
Discussion

In our study, the proportion of monodesethylamodiaquine-resistant isolates was higher than that described in previous studies in Africa, which was between 2 and 7% 15,16,17. Our result can be explained by the high rate of chloroquine resistance in Abidjan area 18,19 and the similar chemical structure between amodiaquine and chloroquine. In Cameroon, IC_{50} values ranging from 25.6 to 115 nM were reported for most of the isolates collected at the time of treatment failure with amodiaquine, indicating that the threshold for monodesethylamodiaquine resistance in vitro might be lower than the usual value of more or equal to 60 nM 20. On this basis, we can say that the rate of decreased sensitivity to monodesethylamodiaquine (i.e IC_{50} > 25 nM) was 28.1% in our study. If the increase in clinical resistance to amodiaquine is confirmed, this situation could compromise the current efficacy of ACT which contains amodiaquine. Indeed, while currently employed ACTs demonstrate excellent clinical efficacy, the history of antimalarial chemotherapy predicts that it is only a matter of time before parasite resistance emerges 21.

All isolates tested in our study were sensitive to quinine, as in the previous in vitro susceptibility tests carried out in Côte d'Ivoire 18,22. These data suggest that quinine still highly effective and confirm the choice to treat severe malaria or treatment failures with this drug. However, we must monitor quinine susceptibility of P. falciparum isolates because of the increasing use of quinine as presumptive treatment for uncomplicated malaria, often without respecting the recommended therapeutic protocol and dosage 23.

This raises the question as to whether drug pressure due to quinine use in urban areas selects parasites with decreased sensitivity to quinine 24. In Senegal, the prevalence of in vitro resistance to quinine was 5% 25, while it was 3% in Comoros 17, 6% in Congo 13 and 8% in Rwanda 16 with intermediate susceptibility to quinine. In Guyana, a reduced P. falciparum sensitivity to quinine was observed in 6/14 isolates tested 26. In Asia, where decreased in vitro susceptibility to quinine was first reported at the beginning of the 1980s in patients living near the Thai-Cambodia border 27 treatment failures with this drug occurred subsequently 5. Thus, it is necessary to evaluate the therapeutic efficacy of quinine in patients.

Despite the uncommon use of mefloquine compared to other antimalarial drugs in Côte d'Ivoire, 15.2% of mefloquine-resistant isolates were observed in our study. The presence of isolates that are naturally less sensitive to mefloquine could partially explain this proportion of resistant isolates. In Senegal, where there was 13% of isolates with reduced susceptibility to mefloquine, prophylactic failures with this drug were previously described 28,29. The same situation could exist in Côte d'Ivoire. Indeed, in this country, mefloquine is one of the drugs recommended to prevent malaria in non-immune populations such as tourists 3. Elsewhere in Africa, in particular in Madagascar and Central African Republic, there were only 2% of in vitro resistance to mefloquine 29,30.

The prevalence of in vitro halofantrine resistance was the highest in our study. In 2002-2003, we found 3 / 11 (27.3%) isolates tested resistant in vitro to halofantrine 31. The data reported in this current study indicate that P. falciparum susceptibility to halofantrine has been stable. From 1994 to 2005, there was an alert issued on halofantrine resistance in French Guiana with a peak of 66% of prevalence of resistance in isolates from 2000 32. In Burkina Faso, where the rate of in vitro resistance to halofantrine was 11.2%, the authors attributed this rate to the presence of isolates naturally resistant, as with mefloquine 23. Indeed, we observed a strongly positive correlation between halofantrine and mefloquine, more than with the other drugs. This positive correlation between two aminoalcohols may be partly explained by their similar chemical structure 33,34. The correlation between monodesethylamodiaquine and aminoalcohols has been previously described 16,34.

A positive correlation between the IC_{50}s of two antimalarial drugs may suggest in vitro cross-resistance 35 although we did not observe cross-resistance with quinine in our study.

Conclusion

In conclusion, our results showed that the in vitro P. falciparum resistance already observed with chloroquine has extended to other antimalarial drugs investigated in this study except for quinine. For quinine, the presence of isolates with reduced susceptibility and correlation with other antimalarial drugs need further investigations.
Acknowledgments
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